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## New Species of *Myxobolus* (Myxosporea: Myxobolidae) Parasites of Fresh Water Fishes in Cameroon (Central Africa)

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**Abstract:** The examination of fresh water fishes captured in the Sangé and Leb mbass rivers (affluent of Nkam at Yabassi and Sanaga at Edéa, respectively) in Cameroon, revealed the presence of three new species of Myxosporean of the genus *Myxobolus* Bütschli, 1882 of which the complete description is given in the present study. *Myxobolus bouixi* sp.n., a gill parasite of *Chrysichthys nigrodigitatus* (Bagridae), forms subspheric spores that measure 11.2 (10.8-12)  $\mu\text{m}$  long  $\times$  10 (9.5-10)  $\mu\text{m}$  broad; the polar capsules measure 4.4 (4-5)  $\times$  3.1 (3-3.5)  $\mu\text{m}$  and contain 5 to 7 coils of polar filament each. *Myxobolus sangei* sp.n., parasite of gills, kidneys and skin in *Brycinus macrolepidotus* (Characidae), forms ovoid spores measuring 10.1 (9 -10.5)  $\mu\text{m}$   $\times$  6.2 (6-6.8)  $\mu\text{m}$ ; its polar capsules are unequal and measure, respectively 6.2 (5.7-7)  $\times$  2.2 (2-3)  $\mu\text{m}$  for the larger and 4.8 (4-5.5)  $\times$  1.7 (1.5-2)  $\mu\text{m}$  for the smaller. *Myxobolus pethericii* sp.n., parasite of the gills, fins, eyes, stomach, liver, intestine, operculum and kidneys of *Ctenopoma petherici* (Anabantidae) forms ovoid spores with a broader anterior end and measuring 12.6 (12-14)  $\times$  7.0 (6.5-7.8)  $\mu\text{m}$ . Its polar capsules measure 5.3 (5-6)  $\times$  1.8 (1.5-2)  $\mu\text{m}$ .

**Key words:** *Myxobolus bouixi* sp.n., *Myxobolus pethericii* sp.n., *Myxobolus sangei* sp.n., fish parasite, Cameroon, Central Africa

### INTRODUCTION

Fishes constitute a favourite biotope for the development of a large number of Protists among which Myxosporeans (Lom and Dykova, 1992; Kent *et al.*, 2001). As essential fish parasites, they remain a potential danger to their hosts. Myxosporean epizootics have been implicated as direct causes of fish mortalities (Schaffer, 1968). In West Africa, Roberts and Sommerville (1982) reported that unidentified myxosporeans might have devastated *Oreochromis niloticus* stocks in cage culture in the Ivory Coast.

Among the 1350 species of Myxozoans known around the world (Kent *et al.*, 2001), the genus *Myxobolus* prevails. Landsberg and Lom (1991) gave the full list of the 444 *Myxobolus* species, with a revision of synonyms. Eiras *et al.* (2005a) in an important synthesis, gave the list of the 744 species of *Myxobolus* described (55.11% of Myxosporean known) with morphometric characteristics of the spores of each species, the host, location in the host and type locality.

In Africa, more than 135 species of Myxosporean are currently known to infect fresh water, brackish and marine fishes (Kostoingué *et al.*, 2001; Abakar-Ousman, 2006).

Fomena and Bouix (1997a) already counted a hundred species in fresh water fishes in that continent. These parasites belong to the genera *Myxobolus* Bütschli, 1882, *Myxobilatus* Davis, 1944; *Henneguya* Thelohan, 1892; *Sphaerospora* Thelohan, 1892; *Chloromyxum* Mingazzini, 1890; *Thelohanellus* Kudo, 1933; *Myxidium* Bütschli, 1882, *Unicauda* Davis, 1944 and *Parahenneguya* Sakiti, 1997.

In Cameroon, data available on Myxosporidians of fresh water fishes are those published by the following authors: Fall *et al.* (2000), Fomena (1995), Fomena and Bouix (1986, 1987, 1994, 1996, 1997a, b and 2000), Fomena *et al.* (1985, 1993 and 1994). These studies have so far revealed the presence of some 62 species belonging to the genera *Myxobolus* (30 species), *Henneguya* (12), *Myxidium* (9), *Thelohanellus* (6), *Sphaerospora* (4) and *Chloromyxum* (1). To effectively carry out fish farming, it is necessary to control not only to master the resulting technical problems, but also pathological problems. Due to the important economic losses caused by some very harmful species, it is important to study in great detail the biodiversity and the biology of Myxosporeans in wild and cultured fishes in order to develop strategies to break off their life cycle.

During a study of Myxosporidia of some fresh water fish species highly consumed by local populations in Cameroon, we found three new parasite species all belonging to the genus *Myxobolus* Bütschli, 1882 of which the complete description is given in the present note. These species are *Myxobolus bouixi* sp.n., found in *Chrysichthys nigrodigitatus* (Lacépède, 1803) (Bagridae), *Myxobolus sangei* sp.n., parasite of *Brycinus macrolepidotus* Valenciennes, 1849 (Characidae), *Myxobolus pethericii* sp.n., parasite of *Ctenopoma petherici* Günther, 1864 (Anabantidae).

## MATERIALS AND METHODS

Fishes examined were captured from November 2006 to April 2007, at Yabassi (in the Sangé River, tributary of Nkam, in the Department of Nkam, Littoral province) and Edéa (in the River Leb Mbass, affluent of Sanaga, in the Department of Sanaga maritime, Littoral province), respectively. Fishes were sampled using gill nets. Systematic position of sampled fishes were taken from Lévêque *et al.* (1990 and 1992). The collected hosts belong to the following families: Anabantidae, Bagridae, Channidae and Characidae. In the laboratory, the fish were examined with the naked eye and then dissected with a stereoscopic microscope Olympus B 061. The following organs were taken and examined: gills, liver, intestine, stomach, kidneys, spleen, gall bladder, urinary bladder. Cysts found were crushed between slide and cover glass and their content identified with the objective 100 x of the microscope Wild M-20. Smears of the kidneys and spleen were examined. Permanent smears of spores were fixed in methanol and stained in May Grünwald-Giemsa. Microphotographies of spores were performed using the Olympus CH-2 microscope. Drawings of fresh spores were carried out using a microscope Wild M-20 equipped with a camera lucida. Measurements were taken on at least 40 fresh spores according to the guidelines proposed by Lom and Arthur (1989).

## RESULTS

### *Myxobolus bouixi* sp.n.

**Description of vegetative stages:** subspherical and polysporous plasmodia were found on the gill arch. A gill arch can carry up to 6 cysts. Measurements of cysts are given in Table 1.

**Description of spores:** In valvular view, spores were subspherical (Fig. 1). Shell valves were thick and smooth. The intercapsular process was absent. The polar capsules were ovoid and of equal size, occupying a nearly 1/3 of

Table 1: Features of the cysts of *Myxobolus* species studied

Source	<i>Myxobolus bouixi</i> sp.n	<i>Myxobolus sangei</i> sp.n	<i>Myxobolus pethericii</i> sp.n
Form of the cysts	Subspherical to elongate	Round	Subspherical to slightly ovoid
Infected organs	Gills	Gills, skin, Kidney	Gills, fins, eyes, stomach wall, liver, small intestine, operculum and kidney
Dimension of the cysts (µm)	750-800× 500-600	up to 270×205	250-850

the spore length (Fig. 1a and b). The polar filament coiled 5 to 7 times almost perpendicular to the polar capsule axis (Fig. 2). The sporoplasm was granular, filling entire extracapsular spore cavity. Measurements of spores are given in Table 2.

**Type host:** *Chrysichthys nigrodigitatus* (Lacépède, 1803) (Bagridae).

**Type locality:** Edéa (in the River Leb Mbass, Littoral province) in Cameroon (Central Africa).

**Location:** Gills

**Prevalence:** 23.52% (4/17)

**Type material:** Glass slides with stained spores (syntype) and cysts preserved in 10% buffered neutral formalin are deposited in the parasitological collection of the Laboratory of General Biology, Faculty of Science, University of Yaounde I, Cameroon (No. Myxo/2007/LBG-006).

**Etymology:** The species is dedicated to Professor Georges Bouix of the University Montpellier II, France.

### *Myxobolus sangei* sp.n.

**Description of vegetative stages:** Subspherical and polysporous cysts were found between the secondary gill lamellae and on the skin. In the kidney, vegetative stages were not found, spores were isolated. Measurements of cysts are given in Table 1.

**Description of spores:** Spores were ovoid with anterior end pointed and posterior end rounded (Fig. 1c). Shell valves were thin and smooth. The two polar capsules were pyriform in shape and of unequal size. The largest polar capsule was lengthened, surpassing the mid-length of the spore and containing 7 to 8 coils of the filament (Fig. 2b). 4 to 5 threads of polar filament were seen in the smallest polar capsule (Fig. 2b). The sporoplasm was granular. Measurements of spores are given in Table 2.

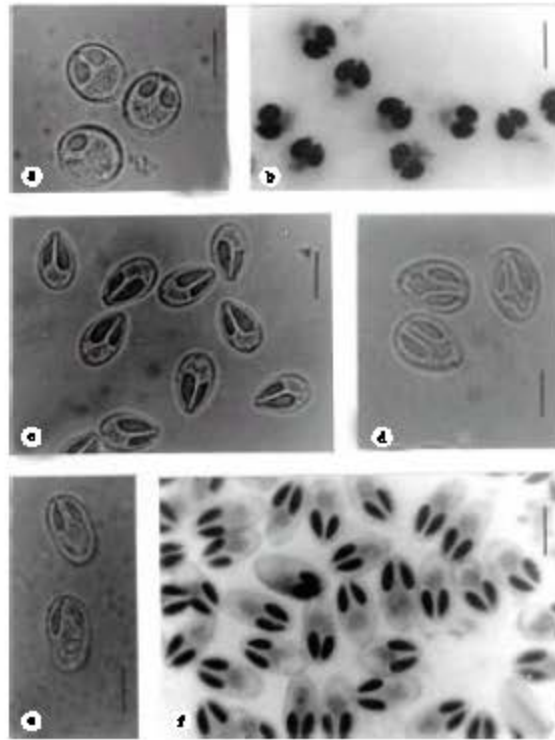


Fig. 1: Microphotographies of spores of *Myxobolus* species studied. *Myxobolus bouxi* sp.n. (a) Fresh spores. Scale bar: 8  $\mu$ m. (b) Spores stained in May Grünwald-Giemsa. Scale bar: 7.5  $\mu$ m. (c) *Myxobolus sangei* sp.n., polar capsules are unequal. Scale bar: 7.5  $\mu$ m. *Myxobolus pethericii* sp.n. 4-5 (d-e) Fresh spores with anterior end broad. Scale bar: 7.8  $\mu$ m. (f) Spores stained in May Grünwald-Giemsa. Scale bar: 9.5  $\mu$ m

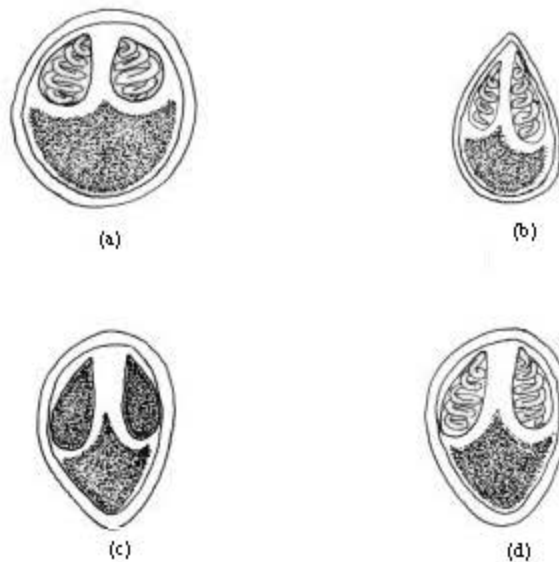


Fig. 2: Line drawings of mature spores. (a) *Myxobolus bouxi* sp.n., parasite of *Chrysichthys nigrodigitatus*. (b) *Myxobolus sangei* sp.n., parasite of *Brycinus macrolepidotus*. (c-d) *Myxobolus pethericii* sp.n., parasite of *Ctenopoma pethericii*. Scale bar: 10  $\mu$ m

Table 2: Features of different spores of *Myxobolus* studied

Properties	<i>Myxobolus bouixi</i> sp.n.	<i>Myxobolus sangei</i> sp.n.	<i>Myxobolus pethericii</i> sp.n.
LS	11.2 (10.8-12)	10.1 (9-10.5)	12.6 (12-14)
WS	10.0 (9.5-10.5)	6.2 (6-6.8)	7.0 (6.5-7.8)
Ratio: LS/WS	1.1	1.6	1.8
PC	=	*	=
LLPC	4.4 (4-5)	6.2 (5.7-7)	5.3 (5-6)
WLPC	3.1 (3-3.5)	2.2 (2-3)	1.8 (1.5-2)
LSPC	-	4.8 (4-5.5)	-
WSPC	-	1.7 (1.5-2)	-
Ratios:			
LLPC/WLPC	1.4	2.8	2.9
LSPC/WSPC	-	2.8	-
LLPC/LS	0.4	0.6	0.4
NC	5-7	7-8*	4-5
		4-5**	

Measurements of parameters are given in (µm). Averages are followed by minimal and maximal values in brackets. LS: Length of the spore; WS: Width of the spore; PC: Relative size of the polar capsules (equal, different); LLPC: Length of the larger polar capsule; WLPC: Width of the larger polar capsule; LSPC: Length of the smaller polar capsule; WSPC: Width of the smaller polar capsule; NC: No. of coils of the polar filament; \*: Referring to the larger polar capsule; \*\*: Referring to the smaller polar capsule

**Type host:** *Brycinus macrolepidotus* Valenciennes, 1849 (Characidae).

**Type locality:** Yabassi (in the River Sangé, Littoral province) in Cameroon (Central Africa).

**Location:** Gill, skin and kidney.

**Prevalence:** All the 4 fish examined were parasitized.

**Type material:** Glass slides with stained spores (syntype) and cysts preserved in 10% buffered neutral formalin are deposited in the parasitological collection of the Laboratory of General Biology, Faculty of Science, University of Yaounde I, Cameroon (No. Myxo/2007/LBG-007).

**Etymology:** The specific name is related to the River Sangé where fish examined were captured.

***Myxobolus pethericii* sp.n.**

**Description of vegetative stages:** Round to slightly ovoid cysts were found located in numerous organs in the host. An individual parasitized host could harbour up to 120 cysts. Spores were diffused in the kidney.

**Description of spores:** In valvular view, mature spores were oblong to oval, with larger and rounded anterior end, the posterior end being narrower (Fig. 1d, e and 2c, d). Two smooth and thick shell valves were visible. The polar capsules were pyriform and of equal size (Fig. 1d-f). They contained four to five coils of polar filament each (Fig. 2d). Measurements of spores are presented in Table 2.

**Type host:** *Ctenopoma petherici* Günther, 1864 (Anabantidae).

**Type locality:** Yabassi (in the River Sangé, Littoral Province) in Cameroon (Central Africa).

**Location:** Numerous organs were affected (Table 1)

**Prevalence:** 100% (15/15).

**Type material:** Glass slides with stained spores (syntype) and cysts preserved in 10% buffered neutral formalin are deposited in the parasitological collection of the Laboratory of General Biology, Faculty of Science, University of Yaounde I, Cameroon (No. Myxo/2007/LBG-008).

**Etymology:** The specific name derives from the mane of the type host.

**DISCUSSION**

***Myxobolus bouixi* sp.n:** Significant differences can be seen when comparing the morphology and spore measurements of *M. bouixi* to that of other African *Myxobolus* species. Concerning the morphology of spores, the most similar are *M. gariepinus* (Reed *et al.*, 2003), *M. nilei* (Faisal and Shalaby, 1987; Fomena and Bouix, 1997), *M. nounensis* (Fomena and Bouix, 2000), *M. gandiolensis* (Fall *et al.*, 2000) *M. nkolyaensis* (Fomena and Bouix, 1994), *M. heterotisi* (Boungou *et al.*, 2006), *M. Kainjiae* (Paperna, 1973; Obiekezie and Okaeme, 1990). The spores of *M. gariepinus* found in the ovaries of *Clarias gariepinus* in Botswana are larger (13.7-15 µm in diameter), with longer polar capsules (6.2 µm). *M. nilei*, a systemic parasite of *Oreochromis niloticus* in Egypt has wider spores (8.20 µm), longer polar capsules (7.5 µm) containing 5 to 6 oblique coils of the polar filament. The spores of *M. nounensis*, a parasite of two Cichlid fishes in Cameroon (*Sarotherodon galilaeus* and *Tilapia mariae*) are larger (14.3×12.8 µm) with a large intercapsular process. In Senegal, *M. gandiolensis* affects kidneys of *Tilapia guineensis*. Its spores are triangular and its polar capsules spherical. Spores of *M. heterotisi*, although of similar dimensions (11.5-13×9.5-10 µm) show a narrower anterior end, longer polar capsules (6-7µm) containing 10 polar filament coils. *M. nkolyaensis*, a parasite of *Barbus jae* in Cameroon, differs from the species in description by its smaller spores (9.0×8.3 µm). Spores of *M. Kainjiae*, a parasite of ovaries in African Cichlid fish are less developed (8.9× 6.6 µm); its polar capsules are smaller (2.4×2.2 µm) and contain 3 to 4 coils of polar filament.

*Myxobolus chrysichthyi*, a gill parasite of *Chrysichthys auratus* in Egypt (Negm-Eldim *et al.*, 1999) has less developed spores (6.2 µm width) with polar capsules containing eight to ten coils of polar filament. In the United Kingdom, *Myxobolus buckei* affects the spinal column in cyprinid fishes (Longshaw *et al.*, 2003). Spores of this species are rounded to ellipsoidal but differs from those of the species in description in being larger (14×11.5 µm); a large intercapsular appendix is present; polar capsules contain 11 to 12 coils of polar filament. *Myxobolus insignis* is a gill parasite of *Semaprochilodus insignis* (an Amazonian teleost) (Eiras *et al.*, 2005b). Despite of a similar overall shape, spores of this species are larger (14.5×11.3 µm); a triangular thickening of the internal face of the wall near posterior end of the polar capsules is present; coils of the polar filament are slightly oblique to the axis of the polar capsule; polar capsules are large, surpassing the mid-length of the spore.

*Myxobolus muelleris* forms small plasmodia in the capillary network of gill lamellae in *Leuciscus cephalus* (Molnár *et al.*, 2006a). The species in description can easily be distinguished from *M. muelleris* because of its larger spores and the absence of the intercapsular process.

It is concluded that the parasite found in *Chrysichthys nigrodigitatus* represents a species not yet described and the name *Myxobolus bouixi* sp.n. is proposed, expression of sympathy to Professor Georges BOUIX of the University Montpellier II, France.

***Myxobolus sangei* sp.n:** Two species of *Myxobolus* are known to infest Characidae fish of the genus *Brycinus* in Africa. These species are *Myxobolus kribiensis*, a systemic parasite of *Brycinus longipinis* in Cameroon (Fomena *et al.*, 1994) and *M. chariensis*, parasite of *Brycinus macrolepidotus* in Chad (Kostoingué *et al.*, 1998). *M. kribiensis* is pyriform and lengthened, with polar capsules slightly unequal. However, it can be discriminated from the species in description by number of features: its spores are larger (20.2-23 µm long); the polar filament is coiled 19 to 28 times in the larger polar capsule.

Whilst the length and the width of *M. chariensis* spores fall within the range of *M. sangei*, its polar capsules are equal and its sutural edge presents markings. The spores of *M. hydrocyni* Kostoingué and Toguebaye (1994), a gill parasite of *Hydrocynus forskalii* (Characidae) in Chad are pyriform with pointed anterior end. These spores are however larger (13-14×8-10 µm) compared to those of the species in description. Its polar capsules are pyriform and equal. *M. scleroperca* Guilford (1963) spores

are larger than *M. sangei* in both length (16.4 µm in average) and polar capsule length (10.8 and 9.8 µm, respectively for the larger and the smaller) compared with 6.2 and 4.8 in our species. In Chad, Abakar-Ousman *et al.* (2006) have described *M. tchadanayei* in the gills of *Citharinus citharus* (Citharinidae). Compared to the species in description, *M. tchadanayei* has larger spores (13.96×8.70 µm); their polar capsules contain a greater number of filament coils (8-9 and 6-7, respectively in the larger and the smaller). *M. fahmii* occurs on the gills of *Barbus bynni* in Egypt (Ali *et al.*, 2002). The overall shape of *M. sangei* resembles *M. fahmii* in having pyriform spore body; measurements of spores are also comparable; the later species however has two polar capsules equal in size. *M. absonus* forms cysts in the opercular cavity of *Pimelodus maculatus* in Brazil (Cellere *et al.*, 2002). This species has much larger spores (15.7×10.2 µm); its polar capsules contain a few number of polar filament coils (5 and 3, respectively in the larger and smaller capsules) compared to 7-8 and 4-5 observed in our species. The spores of *Myxobolus* in description resemble those of *M. etsatsaensis* (Reed *et al.*, 2002), in being elongated, pyriform with blunt anterior end and posterior end rounded. However, the later species differs from our parasite in having longer spores (13 µm in average), polar capsules nearly equal, containing seven to eight coils of the polar filament.

In Malaysia, Molnár *et al.* (2006b) described *Myxobolus baskai* and *M. pangasii* respectively in gills and spleen of *Pangasius hypophthalmus*. The spores of *M. baskai* are ovoid, with rounded posterior end and a slightly tapered anterior end. These spores are however large (13.5-15×10.5-11 µm), with a large intercapsular appendix. Their polar capsules are equal in size. *M. pangasii* presents pyriform and elongated spores which are longer (13.5-15 µm) compared to those of our specimen. The spore wall is thick at the anterior part and polar capsules are elongated but equal in size (constant characteristics).

Considering these differences, we conclude that the parasite in description is new. The name *Myxobolus sangei* sp. n. is proposed referring to the Sangé River in which host fish were captured.

***Myxobolus pethericii* sp.n:** Some species with close general shape can be compared to the present Myxosporean. *Myxobolus ctenopomae* was found in the liver of *Ctenopoma kingsleyae* (Anabantidae) in Benin (Sakiti, 1997). The spores of the species in description are longer compared to those of the later species 12.6 (12-14) µm vs 9.81 (7-11) µm). *M. kingsleyae* Sakiti (1997), a gill parasite of *Ctenopoma kingsleyae* has also shorter spores 10.34 (10-11.5) µm). *M. sarigi* Landsberg (1985), a

systemic parasite in Cichlid fishes in Israel, has shorter (11.3 µm in average) and broader (8.4 µm) spores. Its polar capsules are subspherical. *M. galilaeus* Landsberg (1985) differs markedly by the presence of 3 to 12 folds on its sutural line, our sutural line being not folded. *M. gandioliensis*, a parasite of *Tilapia guineensis* in Senegal (Fall *et al.*, 2000), differs from the species in description in having spherical polar capsules. *M. mbailaoi* (Fomena *et al.*, 2004), a parasite of *Citharinus citharus* in Chad, has ellipsoid spores but with polar capsules unequal in size. *M. amieti* was found in *Ctenopoma nanum* in Cameroon (Fomena *et al.*, 1985). Its spores are ellipsoid to elongate ellipsoid but with slightly pointed anterior end. Its polar capsules are longer compared to those of our species (8.5 vs 5.3 µm) and extend beyond half of the spore. In Egypt, Ali *et al.* (2002) described two Myxosporean with ellipsoidal spores: *Myxobolus caudatus* in the tail fin of *Barbus bynni* and *M. imami* in the kidney of *Lates niloticus*. *M. caudatus* differs from our specimen by its larger spores (16-19.2×11-13.6 µm), its larger polar capsules (7.4×3.8 µm) containing 8 to 9 coils of polar filament, the presence of a relatively large intercapsular process. *M. imami* differs from our species in having shorter spores (10.7 µm in average vs 12.6 µm); five to six notches are present on its sutural wall; its polar capsules contain up to 9 coils of polar filament (vs 4 to 5 in our specimen) slightly oblique along the longitudinal axis of the capsule. *Myxobolus pseudodispar* Gorbunova (1936) (Longshaw *et al.*, 2005) affects juvenile Cyprinids (*Leuciscus cephalus*, *Rutilus rutilus*, *Leuciscus leuciscus*) in UK. Mature spores of this species are ellipsoid to elongate ellipsoid with wider anterior end. Despite of this close general shape, this species has polar capsules most of unequal size.

*Myxobolus* species parasitizing chub are characterized by relatively strict site specificity so that each of them can always be collected from its characteristic location (Molnár *et al.*, 2006a). However, the present study shows that the species in description is a systemic parasite affecting numerous organs in its host.

Considering these arguments, we conclude that the species in description is probably new and the name *Myxobolus pethericii* sp. n. is proposed.

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